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29 MAR 2001

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P010952 GB NJN

2. Patent application number

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29 MAR 2001

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30MAR01 E617995-1 D02246

P01/7700 0.00-0107901.1

3. Full name, address and postcode of the
or of each applicant
(underline all surnames)

Cyclacel Limited
12 St. James Street
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Patents ADP number (if you know it)

07316292004

If the applicant is a corporate body, give
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United Kingdom

4. Title of the invention

Anti-cancer Compounds

5. Name of your agent (if you have one)

D YOUNG & CO

"Address for service" in the United Kingdom
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21 NEW FETTER LANE
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EC4A 1DA

Patents ADP number (if you know it)

59006

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Continuation sheets of this form

Description

25

Claim(s)

7

Abstract

1

Drawing(s)

11/11

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Priority Documents

Translation of Priority Documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

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Request for substantive examination (Patents Form 10/77)

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11.

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Date



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28 Mar 2001

Agents for the Applicants

12. Name and daytime telephone number of person to contact in the United Kingdom

Neil Nachshen

020 7353 4343

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Anti-cancer compounds

The present invention relates to 2-substituted 4-heteroaryl-pyrimidines, their preparation, pharmaceutical compositions containing them, and their use in the 5 treatment of proliferative disorders such as cancer, leukemia, psoriasis and the like.

Introduction and Summary of the Prior art

Certain 4,5,6-Substituted-N-(substituted-phenyl)-2-pyrimidineamines having anti-10 asthmatic properties are disclosed in EP-A-233,461. Certain 4-heteroaryl-N-(3- substituted-phenyl)-2-pyridineamines possessing anti-proliferative properties and inhibiting protein kinases C, epidermal growth factor receptor-associated tyrosine protein kinase (EGF-R-TPK), as well as CDK1/cyclin B have been disclosed in WO95/09847 wherein the exemplified heteroaryl are pyridyl and indolyl.

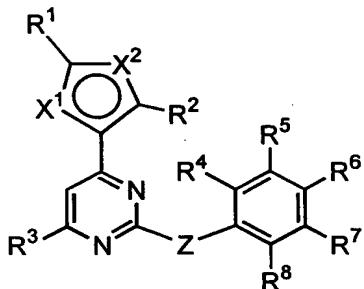
15 J. Med. Chem. (1993) Vol. 36, pages 2716-2725, Paul, R. et al: discloses a further class of phenyl amino-pyrimidines possessing anti-inflammatory activity. These compounds include unsubstituted pyrrol groups, mono-substituted 2-thienyl groups and dimethyl-3-furyl groups at the 4-position of the pyrimidine ring.

20 It is an aim of the present invention to provide a further class of N-phenyl-2-pyrimidine anti-proliferative compounds. The compounds of the present invention have surprisingly been found to not to be inhibitors of protein kinase C. As discussed hereinafter, their activity may be demonstrated by inhibition of cell proliferation in cell lines and/or inhibition of cyclin dependent kinase enzymes.

Summary of the Invention

The first aspect of the present invention relates to compounds of general formula I:

5



wherein:

10 one of X¹ and X² is NH and the other of X¹ and X² is CR⁹;

Z is NH, NHCO, NHSO₂, NHCH₂, CH₂, CH₂CH₂, or CH=CH;

15 R¹, R², R³ and R⁹ are independently H, alkyl, aryl, aralkyl, heterocycle, halogeno, NO₂, CN, OH, alkoxy, aryloxy, NH₂, NH-R', N-(R')(R''), NH-aryl, N-(aryl)₂, COOH, COO-R', COO-aryl, CONH₂, CONH-R', CON-(R')(R''), CONH-aryl, CON-(aryl)₂, SO₃H, SO₂NH₂, CF₃, CO-R', or CO-aryl, wherein alkyl, aryl, aralkyl and heterocycle groups may be further substituted with one or more groups selected from halogeno, NO₂, CN, OH, O-methyl, NH₂, COOH, CONH₂ and CF₃;

20

R⁴, R⁵, R⁶, R⁷, and R⁸ are independently from each other H, substituted or unsubstituted lower alkyl, halogeno, NO₂, CN, OH, substituted or unsubstituted alkoxy, NH₂, NH-R', N-(R')(R''), COOH, COO-R', CONH₂, CONH-R', CON-(R')(R''), SO₃H, SO₂NH₂, or CF₃;

25

wherein R' and R'' are each independently alkyl groups that may be the same or different;

with the proviso that when R¹ and R² are H, X¹ is NH, X² is CH, and R³ is H, the phenyl group is not

- 5 unsubstituted,
- 4-ethyl,
- 3-methyl,
- 3-(1,1,2,2-tetrafluoroethoxy),
- 3,4,5-trimethoxy,
- 10 when the other groups R⁴-R⁸ are H;

and pharmaceutically acceptable salts thereof.

Description of the Preferred Embodiments

- 15 As used herein the term "alkyl" includes both straight chain and branched alkyl groups having from 1 to 8 carbon atoms, e.g. methyl, ethyl propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl etc and the term "lower alkyl" is similarly used for groups having from 1 to 4 carbon atoms.
- 20 The term "aryl" is used to include groups having from 6 to 10 carbon atoms, e.g. phenyl, naphthyl etc.

The term "aralkyl" is used as a conjunction of the terms alkyl and aryl as given above.

- 25 Preferred compounds of formula I are those bearing a mono- or di-substituted pyrrol radical, attached to the pyrimidine ring through one of the ring carbon atoms. Preferably, the pyrrol radical is a pyrrol-3-yl group.

The pyrrol group is substituted by R¹, R² and R⁹. Preferably, R¹, R² and where appropriate R⁹ are each independently selected from H, alkyl, aryl, aralkyl, halogeno, NO₂, CN, OH, alkoxy, aryloxy, NH₂, NH-R', N-(R')(R''), COOH, COO-R', CONH₂, CONH-R', CON-(R')(R''), SO₃H, SO₂NH₂, CF₃, and CO-R' wherein alkyl, aryl and aralkyl groups may be further substituted with one or more groups selected from halogeno, NO₂, CN, OH, O-methyl, NH₂, COOH, CONH₂ and CF₃.

More preferably, R¹, R² and R⁹ are each independently selected from H, halogeno or C₁₋₄ alkyl group.

10

Preferably, R¹ is H.

Even more preferably, R¹ is H, and R² and R⁹ are both chloro or methyl.

15

The group Z is preferably NH, NHSO₂ or NHCH₂, most preferably NH.

The phenyl substituents R⁴-R⁸ may be selected from H, halogeno, amino, nitro, alkoxy such as methoxy, carbamoyl, sulfamyl and C₁₋₄ alkyl each optionally substituted by OH, or halogen such as trifluoromethyl, etc.

20

More preferably, the phenyl substituents R⁴-R⁸ are selected from H, F, NH₂, NO₂, OH, Cl, Br, I, CN, CH₂OH, CF₃ and OMe.

25

Thus, particularly preferred embodiments include 2-[N-(phenyl)]-4-(2,4-dimethylpyrrol-3-yl)pyrimidineamines in which the phenyl group is 2-, 3- or 4-substituted by at least one of H, F, NH₂, NO₂, OH, Cl, Br, I, CN, CH₂OH, CF₃ or OMe.

Even more preferably, the phenyl group is mono-substituted by F, NH₂, NO₂, OH, Cl, Br, I, CH₂OH, CN, CF₃ or OMe at any of the 2,3 or 4-positions, or di-substituted by 2,4-difluoro, 3,5-difluoro, 3,4-difluoro, 2,4-dichloro, 3,5-dichloro, 3,4-dichloro or 4-chloro-3-trifluoromethyl.

5

Most preferably, the compounds of the present invention are selected from:

{3-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-phenyl}-methanol,

3-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-benzonitrile,

4-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-benzonitrile,

10 [4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-[4-fluoro-phenyl]-amine,

[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-[3-nitro-phenyl]-amine,

[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-[4-iodo-phenyl]-amine,

(3,4-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,

(4-Chloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,

15 (3,5-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,

4-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-phenol,

3-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-phenol,

(2,4-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,

(2,4-Dichloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,

20 (4-Chloro-3-trifluoromethyl-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,

[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-[4-trifluoromethyl-phenyl]-amine,

[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-[3-trifluoromethyl-phenyl]-amine,

(3-Chloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine.

25

The structures of the above-mentioned compounds are illustrated in Figure 1.

Particularly preferred compounds observed to be CDK inhibitors include the following:

[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-4-fluoro-phenyl)-amine,
[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-3-nitro-phenyl)-amine,
[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-4-iodo-phenyl)-amine,
4-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-benzonitrile,
5 (3,4-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
(3-Chloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
(3,5-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
3-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-phenol,
(4-Chloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
10 4-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-phenol,
(2,4-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-4-trifluoromethyl-phenyl)-amine,
and
[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-3-trifluoromethyl-phenyl)-amine.
15 The following compounds are observed to be particularly effective anti-proliferative agents, as demonstrated by cell-based assays:
(2,4-Dichloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine, and
(4-Chloro-3-trifluoromethyl-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine.
20 The compounds of formula I have been found to possess anti-proliferative activity and are therefore believed to be of use in the treatment of proliferative disorders such as cancers, leukaemias and other disorders associated with uncontrolled cellular proliferation such as psoriasis and restenosis. As defined herein, an anti-proliferative effect within the scope of the present invention may be demonstrated by the ability to inhibit cell proliferation in an *in vitro* whole cell assay, for example using any of the cell lines A549, HT29, Saos-2, HeLa or MCF-7, or by showing inhibition of a CDK enzyme (such as CDK2 or CDK4) in an appropriate assay. These assays, including methods for their performance, are described in more detail in Example 3. Using such

cell line and enzymes assays it may be determined whether a compound is anti-proliferative in the context of the present invention.

Without wishing to be bound by theory, the compounds of the present invention are

5 believed to exert their anti-proliferative effect in a non-protein kinase C (PKC) dependent manner. Many of the compounds inhibit cyclin-dependent kinase enzymes (CDKs) that have been shown to be involved in cell cycle control. These CDKs include CDK2 and CDK4 and particularly their respective interactions with cyclin E and cyclin D1. These compounds of the present invention are further believed to be

10 advantageous in being selective for CDK enzymes implicated in proliferative diseases. By the term "selective" it is meant that although possible having some inhibitory effect on another enzyme (such as PKC), the compound is preferentially effective against an enzyme implicated in proliferative diseases.

15 The compounds of the invention may inhibit any of the steps or stages in the cell cycle, for example, formation of the nuclear envelope, exit from the quiescent phase of the cell cycle (G0), G1 progression, chromosome decondensation, nuclear envelope breakdown, START, initiation of DNA replication, progression of DNA replication, termination of DNA replication, centrosome duplication, G2 progression, activation of

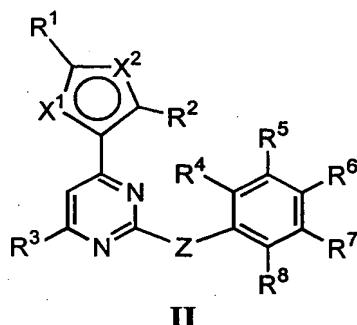
20 mitotic or meiotic functions, chromosome condensation, centrosome separation, microtubule nucleation, spindle formation and function, interactions with microtubule motor proteins, chromatid separation and segregation, inactivation of mitotic functions, formation of contractile ring, and cytokinesis functions. In particular, the compounds of the invention may influence certain gene functions such as chromatin

25 binding, formation of replication complexes, replication licensing, phosphorylation or other secondary modification activity, proteolytic degradation, microtubule binding, actin binding, septin binding, microtubule organising centre nucleation activity and binding to components of cell cycle signalling pathways.

A further embodiment of the present invention therefore relates to the use of one or more compounds of formula I in the treatment of proliferative disorders. Preferably, the proliferative disorder is a cancer or leukaemia. The term proliferative disorder is used herein in a broad sense to include any disorder that requires control of the cell 5 cycle, for example cardiovascular disorders such as restenosis and cardiomyopathy, auto-immune disorders such as glomerulonephritis and rheumatoid arthritis, dermatological disorders such as psoriasis, anti-inflammatory, anti-fungal, antiparasitic disorders such as malaria, emphysema and alopecia. In these disorders, the compounds of the present invention may induce apoptosis or maintain stasis 10 within the desired cells as required.

In a particularly preferred embodiment, the invention relates to the use of one or more compounds of formula I in the treatment of a CDK dependent or sensitive disorder. CDK dependent disorders are associated with an above normal level of activity of one 15 or more CDK enzymes. Such disorders preferably associated with an abnormal level of activity of CDK2 and/or CDK4. A CDK sensitive disorder is a disorder in which an aberration in the CDK level is not the primary cause, but is downstream of the primary metabolic aberration. In such scenarios, CDK2 and/or CDK4 can be said to be part of the sensitive metabolic pathway and CDK inhibitors may therefore be active 20 in treating such disorders. Such disorders are preferably cancer or leukaemic disorders.

A second aspect of the present invention relates to the use of a compound of formula



wherein:

one of X^1 and X^2 is NH and the other of X^1 and X^2 is CR^9 ;

5 Z is NH, NHCO, $NHSO_2$, $NHCH_2$, CH_2 , CH_2CH_2 , or $CH=CH$;

R^1 , R^2 , R^3 and R^9 are independently H, alkyl, aryl, aralkyl, heterocycle, halogeno, NO_2 , CN, OH, alkoxy, aryloxy, NH_2 , $NH-R'$, $N-(R')(R'')$, $NH-aryl$, $N-(aryl)_2$, COOH, $COO-R'$, $COO-aryl$, $CONH_2$, $CONH-R'$, $CON-(R')(R'')$, $CONH-aryl$, $CON-(aryl)_2$,
10 SO_3H , SO_2NH_2 , CF_3 , $CO-R'$, or $CO-aryl$, wherein alkyl, aryl, aralkyl and heterocycle groups may be further substituted with one or more groups selected from halogeno, NO_2 , CN, OH, O-methyl, NH_2 , COOH, $CONH_2$ and CF_3 ;

R^4 , R^5 , R^6 , R^7 , and R^8 are independently from each other H, substituted or
15 unsubstituted lower alkyl, halogeno, NO_2 , CN, OH, substituted or unsubstituted alkoxy, NH_2 , $NH-R'$, $N-(R')(R'')$, COOH, $COO-R'$, $CONH_2$, $CONH-R'$, $CON-(R')(R'')$, SO_3H , SO_2NH_2 , or CF_3 ;

wherein R' and R'' are each independently alkyl groups that may be the same or
20 different;

with the proviso that when R^1 and R^2 are H, X^1 is NH, X^2 is CH , and R^3 is H, the phenyl group is not

3-(1,1,2,2-tetrafluoroethoxy), or

25 3,4,5-trimethoxy,

when the other groups R^4-R^8 are H;

and pharmaceutically acceptable salts thereof;

in the manufacture of a medicament for use in the treatment of a proliferative disease.

The term "proliferative disorder" has been previously discussed and the same definition applies to the second aspect of the invention.

5 The preferred embodiments of this further aspect of the invention are identical to those described above in respect of the first aspect.

In a particularly preferred embodiment, the one or more compounds of the invention are administered in combination with one or more other anticancer agents. In such cases, the compounds of the invention may be administered consecutively, 10 simultaneously or sequentially with the one or more other anticancer agents.

15 As used herein the phrase "manufacture of a medicament" includes the use of a compound of formula I directly as the medicament in addition to its use in a screening programme for further anti-proliferative agents or in any stage of the manufacture of such a medicament.

The compounds of the present invention (first and seconds aspects) can be present as salts or esters, in particular pharmaceutically acceptable salts or esters.

20 Pharmaceutically acceptable salts of the compounds of the invention (first and seconds aspects) include suitable acid addition or base salts thereof. A review of suitable pharmaceutical salts may be found in Berge et al, *J Pharm Sci*, 66, 1-19 (1977). Salts are formed, for example with strong inorganic acids such as mineral acids, e.g. sulphuric acid, phosphoric acid or hydrohalic acids; with strong organic 25 carboxylic acids, such as alkanecarboxylic acids of 1 to 4 carbon atoms which are unsubstituted or substituted (e.g., by halogen), such as acetic acid; with saturated or unsaturated dicarboxylic acids, for example oxalic, malonic, succinic, maleic, fumaric, phthalic or tetraphthalic; with hydroxycarboxylic acids, for example ascorbic, glycolic, lactic, malic, tartaric or citric acid; with aminoacids, for example aspartic or

glutamic acid; with benzoic acid; or with organic sulfonic acids, such as (C₁-C₄)-alkyl- or aryl-sulfonic acids which are unsubstituted or substituted (for example, by a halogen) such as methane- or p-toluene sulfonic acid.

- 5 Esters are formed either using organic acids or alcohols/hydroxides, depending on the functional group being esterified. Organic acids include carboxylic acids, such as alkanecarboxylic acids of 1 to 12 carbon atoms which are unsubstituted or substituted (e.g., by halogen), such as acetic acid; with saturated or unsaturated dicarboxylic acid, for example oxalic, malonic, succinic, maleic, fumaric, phthalic or tetraphthalic; with 10 hydroxycarboxylic acids, for example ascorbic, glycolic, lactic, malic, tartaric or citric acid; with aminoacids, for example aspartic or glutamic acid; with benzoic acid; or with organic sulfonic acids, such as (C₁-C₄)-alkyl- or aryl-sulfonic acids which are unsubstituted or substituted (for example, by a halogen) such as methane- or p-toluene sulfonic acid. Suitable hydroxides include inorganic hydroxides, such as sodium 15 hydroxide, potassium hydroxide, calcium hydroxide, aluminium hydroxide. Alcohols include alkanealcohols of 1-12 carbon atoms which may be unsubstituted or substituted, e.g. by a halogen).

In all aspects of the present invention previously discussed, the invention includes, 20 where appropriate all enantiomers and tautomers of compounds of formula I. The man skilled in the art will recognise compounds that possess an optical properties (one or more chiral carbon atoms) or tautomeric characteristics. The corresponding enantiomers and/or tautomers may be isolated/prepared by methods known in the art.

- 25 The invention furthermore relates to the compounds of or of use in the present invention (first and seconds aspects) in their various crystalline forms, polymorphic forms and (an)hydrorous forms. It is well established within the pharmaceutical industry that chemical compounds may be isolated in any of such forms by slightly

varying the method of purification and or isolation form the solvents used in the synthetic preparation of such compounds.

The invention further includes the compounds (first and seconds aspects) of or of use

5 in the present invention in prodrug form. Such prodrugs are generally compounds of formula I wherein one or more appropriate groups have been modified such that the modification may be reversed upon administration to a human or mammalian subject. Such reversion is usually performed by an enzyme naturally present in such subject, though it is possible for a second agent to be administered together with such a

10 prodrug in order to perform the reversion in vivo. Examples of such modifications include ester (for example, any of those described above), wherein the reversion may be carried out be an esterase etc. Other such systems will be well known to those skilled in the art.

15 The present invention also encompasses pharmaceutical compositions comprising the compounds of the invention (first and seconds aspects). In this regard, and in particular for human therapy, even though the compounds of the present invention (including their pharmaceutically acceptable salts, esters and pharmaceutically acceptable solvates) can be administered alone, they will generally be administered in

20 admixture with a pharmaceutical carrier, excipient or diluent selected with regard to the intended route of administration and standard pharmaceutical practice.

Thus, the present invention also relates to pharmaceutical compositions comprising one or more compounds of formula I or II or pharmaceutically acceptable salts or esters thereof, together with at least one pharmaceutically acceptable excipient, diluent or carrier.

By way of example, in the pharmaceutical compositions of the present invention, the compounds of the invention may be admixed with any suitable binder(s), lubricant(s),

suspending agent(s), coating agent(s), and/or solubilising agent(s). Examples of such suitable excipients for the various different forms of pharmaceutical compositions described herein may be found in the "Handbook of Pharmaceutical Excipients, 2nd Edition, (1994), Edited by A Wade and PJ Weller.

5

The pharmaceutical compositions of the present invention may be adapted for oral, rectal, vaginal, parenteral, intramuscular, intraperitoneal, intraarterial, intrathecal, intrabronchial, subcutaneous, intradermal, intravenous, nasal, buccal or sublingual routes of administration.

10

For oral administration, particular use is made of compressed tablets, pills, tablets, gellules, drops, and capsules. Preferably, these compositions contain from 1 to 250 mg and more preferably from 10-100 mg, of active ingredient per dose.

15

Other forms of administration comprise solutions or emulsions which may be injected intravenously, intraarterially, intrathecally, subcutaneously, intradermally, intraperitoneally or intramuscularly, and which are prepared from sterile or sterilisable solutions. The pharmaceutical compositions of the present invention may also be in form of suppositories, pessaries, suspensions, emulsions, lotions, ointments, creams, 20 gels, sprays, solutions or dusting powders.

25

An alternative means of transdermal administration is by use of a skin patch. For example, the active ingredient can be incorporated into a cream consisting of an aqueous emulsion of polyethylene glycols or liquid paraffin. The active ingredient can also be incorporated, at a concentration of between 1 and 10% by weight, into an ointment consisting of a white wax or white soft paraffin base together with such stabilisers and preservatives as may be required.

Injectable forms may contain between 10 - 1000 mg, preferably between 10 - 250 mg, of active ingredient per dose.

5 Compositions may be formulated in unit dosage form, i.e., in the form of discrete portions containing a unit dose, or a multiple or sub-unit of a unit dose.

A person of ordinary skill in the art can easily determine an appropriate dose of one of the instant compositions to administer to a subject without undue experimentation. Typically, a physician will determine the actual dosage which will be most suitable for 10 an individual patient and it will vary with the age, weight and response of the particular patient. The dosages disclosed herein are exemplary of the average case. There can of course be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

15 In an exemplary embodiment, one or more doses of 10 to 150 mg/day will be administered to the patient for the treatment of malignancy.

20 The pharmaceutical compositions of the invention may further comprise one or more additional anticancer agents, for example, existing anticancer drugs available on the market.

Anticancer drugs in general are more effective when used in combination. In particular, combination therapy is desirable in order to avoid an overlap of major toxicities, mechanism of action and resistance mechanism(s). Furthermore, it is also 25 desirable to administer most drugs at their maximum tolerated doses with minimum time intervals between such doses. The major advantages of combining chemotherapeutic drugs are that it may promote additive or possible synergistic effects through biochemical interactions and also may decrease the emergence of resistance in early tumor cells which would have been otherwise responsive to initial chemotherapy

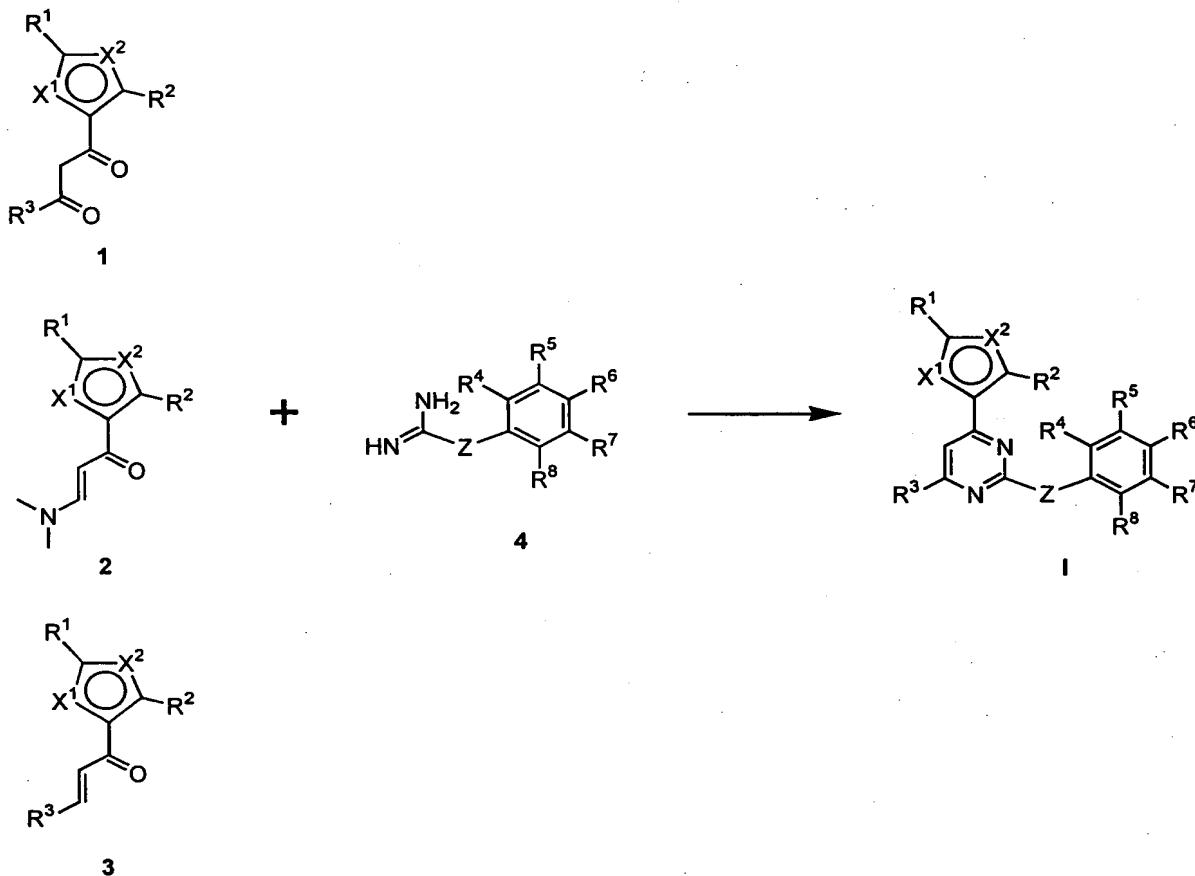
with a single agent. An example of the use of biochemical interactions in selecting drug combinations is demonstrated by the administration of leucovorin to increase the binding of an active intracellular metabolite of 5-fluorouracil to its target, thymidylate synthase, thus increasing its cytotoxic effects.

5

Numerous combinations are used in current treatments of cancer and leukemia. A more extensive review of medical practices may be found in "Oncologic Therapies" edited by E. E. Vokes and H. M. Golomb, published by Springer.

10 Beneficial combinations may be suggested by studying the growth inhibitory activity of the test compounds with agents known or suspected of being valuable in the treatment of a particular cancer initially or cell lines derived from that cancer. This procedure can also be used to determine the order of administration of the agents, *i.e.* before, simultaneously, or after delivery. Such scheduling may be a feature of all the
15 cycle acting agents identified herein.

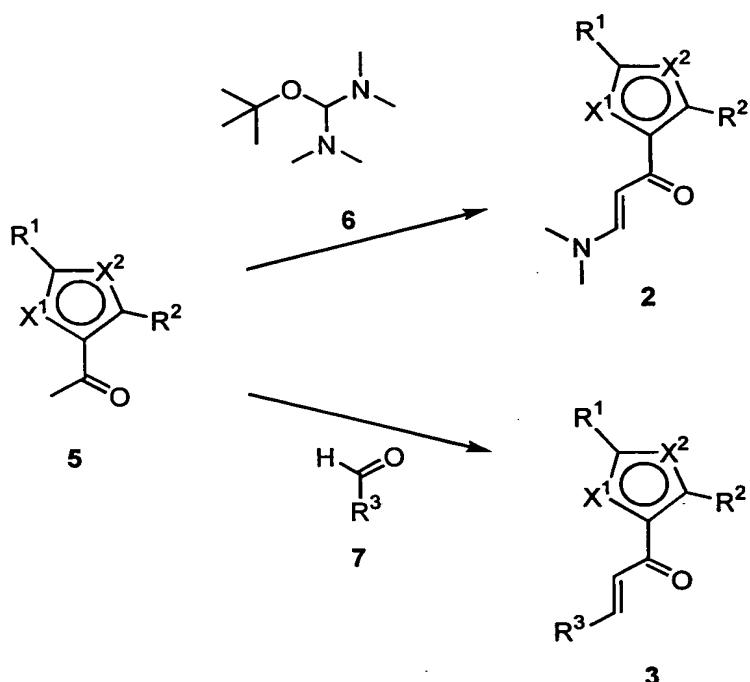
20 The compounds of this invention (I) can be synthesised, for example, by an adaptation of the Traube synthesis (A.R. Katritzky, I. Taher, *Can. J. Chem.* 1986, **64**, 2087 and references cited therein), *i.e.* by condensation between 1,3-dicarbonyl compounds **1** or acrylates **2** or **3**, and amidine **4**, as shown in *Scheme 1*.



Scheme 1

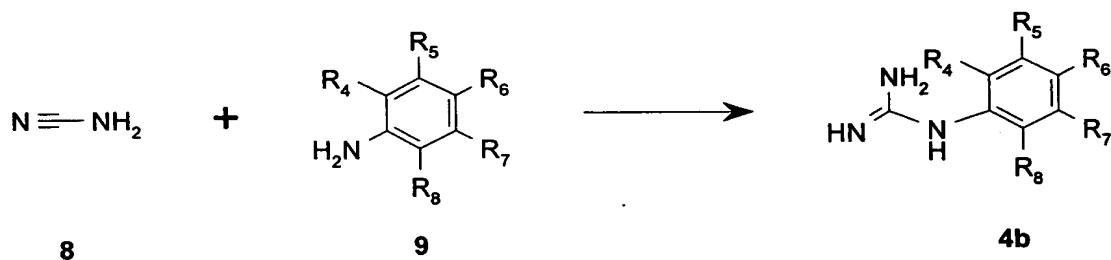
5 The dicarbonyl compounds I in turn can be prepared by many methods known in the art (J. March, *In: Advanced Organic Chemistry: Reactions, Mechanism, and Structure*, 4th Ed., John Wiley & Sons, Inc., New York, 1992, p. 1283). Acrylates 2 and 3, which are particularly suitable for the purposes of this invention, are obtained from heterocyclic methyl ketones 5 by condensation with *tert*-

10 butoxybis(dimethylamino)methane 6 (*Scheme 2*).



The diamino compounds **4** will be amidines **4a** or guanidines **4b**, depending on the definition of Z in general structure **I**. Amidines ($\text{HN}=\text{CRNH}_2$) can be obtained from readily available amine precursors by condensation with *e.g.* ketenimines, or by addition of ammonia to suitable nitriles or imidates. Guanidines **4b** (*Scheme 3*) can be elaborated by a number of methods known in the art. For the purposes of this invention, the most useful route is amination of cyanamide **8** with anilines **9**.

10



Alternatively, compounds of general structure I can be obtained from suitable pyrimidine precursors directly, *e.g.* from 2,4-disubstituted (halogen, amine, *etc.*) pyrimidines by successive substitution reactions.

5 The present invention will now be described by way of example and with reference to the following figure:

Figure 1 shows the chemical structure of compounds 1-17 according to the invention.

10 **Examples**

Abbreviations

LC-MS, liquid chromatography-mass spectrometry; NMR, nuclear magnetic resonance spectroscopy; r.t. room temperature; PE, petroleum ether (40-60 °C boiling fraction); DMSO, dimethylsulfoxide.

General

NMR spectra were recorded using a Bruker DPX-300 instrument. Chemical shifts are reported in ppm (δ) from tetramethylsilane. EM Kieselgel 60 (0.040-0.063 mm) was 20 used for flash column chromatography. Melting points were determined with a LEICA testo-720 electrothermometer and are uncorrected. Compound numbers are shown in brackets, where appropriate.

Example 1

25 ***3-Dimethylamino-1-(2,4-dimethyl-1H-pyrrol-3-yl)-propanone***

A mixture of 3-acetyl-2,4-dimethylpyrrole (2 g, 15 mmol) in 5 mL of *tert*-butoxy-bis (dimethylamino)methane was heated at 100 °C for 22 h. The precipitates of the reaction mixture were slurried in EtOAc/PE with chilling. The crude product was filtered, washed with EtOAc/PE and dried *in vacuo* to afford the title compound as a

purple solid (2.6 g). $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 2.25 (s, 6H, CH_3), 2.45 (s, 6H, CH_3), 5.46 (d, 1H, J = 12.6 Hz, CH), 6.35 (s, 1H, pyrrole- H), 7.63 (d, 1H, J = 12.6 Hz, CH).

5 **Example 2 [1]**

[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(4-fluoro-phenyl)-amine

To a mixture of 3-dimethylamino-1-(2,4-dimethyl-1H-pyrrol-3-yl)-propanone (1 mmol, 0.19 g) and 4-fluorophenyl guanidine nitrate (2 mmol, 0.44 g) in 5 mL of 2-methoxyethanol was added 40 mg NaOH. The reaction mixture was heated at 100-120 10 $^{\circ}\text{C}$ under N_2 for 6 h. The solvent was evaporated to dryness and the residue was purified by flash chromatography, using EtOAc/PE (1:2, v/v) to elute the product as a brown solid. Recrystallisation from EtOAc/PE yielded the title compound (174 mg, 62 %) as brown crystals. $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 2.21 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 6.33 (s, 1H, pyrrole- H), 6.73 (d, 1H, J = 5.3 Hz, pyrimidinyl- H), 7.00 (m, 2H, 15 Ph-H), 7.79 (m, 2H, Ph-H), 8.28 (d, 1H, J = 5.3 Hz, pyrimidinyl- H), 9.16 (s, 1H, NH), 10.59 (s, 1H, NH).

The following compounds were prepared in a manner analogous to that described above:

20

[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(3-nitro-phenyl)-amine [2]

Yellow-orange solid. M.p. 197-199 $^{\circ}\text{C}$. LC-MS: m/z = 310 (M+1). $\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_2$ requires C, 62.12; H, 4.89; N, 22.64; found C, 62.61; H, 4.99; N, 22.20. $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 2.71 (d, 6H, CH_3), 7.05 (d, 1H, J = 5.3 Hz, pyrimidinyl- H), 7.47 (m, 2H, Ph-H), 7.78 (m, 1H, Ph-H), 7.81 (s, 1H, Ar- H), 8.07 (m, 1H, Ph-H), 8.51 (d, 1H, J = 5.3 Hz, pyrimidinyl- H), 8.99 (sbr, 1H, NH), 9.91 (sbr, 1H, NH).

[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(4-iodo-phenyl)-amine [3] $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 2.26 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 6.80 (d, 1H, J = 5.3 Hz,

pyrimidinyl-*H*), 7.47 (m, 2H, Ph-*H*), 7.57 (m, 2H, Ph-*H*), 7.23 (s, 1H, pyrimidinyl-*H*), 8.32 (d, 1H, *J* = 5.3 Hz, pyrimidinyl-*H*).

(3,4-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine [6]

5 $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 2.23 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 6.42 (s, 1H, pyrimidinyl-*H*), 6.77 (d, 1H, *J* = 5.3 Hz, pyrimidinyl-*H*), 7.11 (m, 1H, Ph-*H*), 7.41 (m, 1H, Ph-*H*), 8.05 (m, 1H, Ph-*H*), 8.29 (d, 1H, *J* = 5.3 Hz, pyrimidinyl-*H*), 9.21 (s, 1H, Ph-*H*), 10.46 (sbr, 1H, *NH*).

10 *(4-Chloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine [10]*

$^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 2.19 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 6.48 (s, 1H, pyrimidinyl-*H*), 6.82 (d, 1H, *J* = 5.3 Hz, pyrimidinyl-*H*), 7.31 (m, 2H, Ph-*H*), 7.84 (m, 2H, Ph-*H*), 8.34 (d, 1H, *J* = 5.3 Hz, pyrimidinyl-*H*), 9.45 (s, 1H, Ph-*H*), 10.72 (sbr, 1H, *NH*).

15

(3,5-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine [8]

$^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 2.25 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 6.45 (m, 2H, pyrimidinyl-*H* & Ph-*H*), 6.80 (d, 1H, *J* = 5.5 Hz, pyrimidinyl-*H*), 7.46 (m, 1H, Ph-*H*), 8.31 (d, 1H, *J* = 5.5 Hz, pyrimidinyl-*H*).

20

4-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-phenol [11]

$^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 2.23 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 6.44 (s, 1H, pyrimidinyl-*H*), 6.76 (m, 3H, pyrimidinyl-*H* & Ph-*H*), 7.39 (m, 2H, Ph-*H*), 8.17 (d, 1H, *J* = 5.3 Hz, pyrimidinyl-*H*).

25

3-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-phenol [9]

$^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 2.26 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 6.46 (s, 1H, pyrimidinyl-*H*), 6.84 (d, 1H, *J* = 5.4 Hz, pyrimidinyl-*H*), 7.07 (m, 2H, Ph-*H*), 7.32 (m, 1H, Ph-*H*), 8.25 (d, 1H, *J* = 5.3 Hz, pyrimidinyl-*H*).

(2,4-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine [12]

¹H-NMR (300 MHz, CDCl₃) δ 2.10 (s, 3H, CH₃), 2.36 (s, 3H, CH₃), 6.34 (s, 1H, pyrimidinyl-H), 6.77 (d, 1H, J = 5.3 Hz, pyrimidinyl-H), 7.06 (m, 1H, Ph-H), 7.66 (m, 1H, Ph-H), 8.25 (d, 1H, J = 5.3 Hz, pyrimidinyl-H), 8.71 (s, 1H, Ph-H), 10.70 (sbr, 1H, NH).

(2,4-Dichloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine [13]

¹H-NMR (300 MHz, CDCl₃) δ 2.18 (s, 3H, CH₃), 2.38 (s, 3H, CH₃), 6.51 (s, 1H, pyrimidinyl-H), 6.90 (d, 1H, J = 5.5 Hz, pyrimidinyl-H), 7.46 (m, 1H, Ph-H), 7.71 (m, 1H, Ph-H), 8.05 (m, 1H, Ph-H), 8.36 (d, 1H, J = 5.3 Hz, pyrimidinyl-H), 8.49 (s, 1H, Ph-H), 10.80 (sbr, 1H, NH).

(4-Chloro-3-trifluoromethyl-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine [14]

¹H-NMR (300 MHz, CDCl₃) δ 2.19 (s, 3H, CH₃), 2.42 (s, 3H, CH₃), 6.50 (s, 1H, pyrimidinyl-H), 6.89 (d, 1H, J = 5.3 Hz, pyrimidinyl-H), 7.61 (m, 1H, Ph-H), 8.08 (m, 1H, Ph-H), 8.41 (m, 2H, Ph-H & pyrimidinyl-H), 9.79 (s, 1H), 10.80 (sbr, 1H, NH).

[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-[4-trifluoromethyl-phenyl]-amine

[15]

¹H-NMR (300 MHz, CDCl₃) δ 2.26 (s, 3H, CH₃), 2.49 (s, 3H, CH₃), 6.56 (s, 1H, pyrimidinyl-H), 6.94 (d, 1H, J = 5.3 Hz, pyrimidinyl-H), 7.67 (m, 1H, Ph-H), 8.09 (m, 2H, Ph-H), 8.45 (d, 1H, J = 5.3 Hz, pyrimidinyl-H), 9.82 (s, 1H, NH).

[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-[3-trifluoromethyl-phenyl]-amine

[16]

¹H-NMR (300 MHz, CDCl₃) δ 2.26 (s, 3H, CH₃), 2.48 (s, 3H, CH₃), 6.56 (s, 1H, pyrimidinyl-H), 6.92 (d, 1H, J = 5.3 Hz, pyrimidinyl-H), 7.29 (m, 1H, Ph-H), 7.55 (m,

1H, Ph-*H*), 8.03 (m, 1H, Ph-*H*), 8.43 (m, 2H, pyrimidinyl-*H* & Ph-*H*), 9.73 (s, 1H, NH), 10.83 (sbr, 1H, NH).

(3-Chloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine [7]

5 ¹H-NMR (300 MHz, CDCl₃) δ 2.21 (s, 3H, CH₃), 2.44 (s, 3H, CH₃), 6.51 (s, 1H, pyrimidinyl-*H*), 6.85 (d, 1H, *J* = 5.3 Hz, pyrimidinyl-*H*), 6.94 (m, 1H, Ph-*H*), 7.28 (m, 1H, Ph-*H*), 8.19 (s, 1H, Ph-*H*), 8.37 (d, 1H, *J* = 5.3 Hz, pyrimidinyl-*H*), 9.55 (s, 1H, NH), 10.78 (sbr, 1H, NH).

10 Example 3

Kinase specificity of selected compound

Selected compounds from the above examples were investigated for their kinase selectivity. A panel of protein kinases, including the CDKs relevant to the present invention, as well as a representative number of functionally unrelated kinases, were 15 used.

Assays for CDK4/Cyclin D1, CDK2/Cyclin E, CDK1/Cyclin B kinase may be carried out by monitoring phosphorylation of GST-Rb in an appropriate system. Thus, GST-Rb phosphorylation, induced by CDK4/Cyclin D1, CDK2/Cyclin E or CDK1/Cyclin 20 B is determined by incorporation of radio-labeled phosphate in GST-Rb(772-928) using radiolabelled ATP in 96-well format *in vitro* kinase assay. The phosphorylation reaction mixture (total volume 40 μ l) consisted of 50 mM HEPES pH 7.4, 20 mM MgCl₂, 5 mM EGTA, 2 mM DTT, 20 mM β -glycerophosphate, 2 mM NaF, 1 mM Na₃VO₄, Protease Inhibitors Cocktail (Sigma, see above), BSA 0.5mg/ml, 1 μ g 25 purified enzyme complex, 10 μ l of GST-Rb-Sepharose beads, 100 μ M ATP, 0.2 μ Ci ³²P-ATP. The reaction is carried out for 30 min at 30°C at constant shaking. At the end of this period 100 μ l of 50 mM HEPES, pH 7.4 and 1 mM ATP is added to each well and the total volume transferred onto GFC filtered plate. The plate is washed 5 times with 200 μ l of 50 mM HEPES, pH 7.4 and 1 mM ATP. To each well were

added 50 μ l scintillant liquid and the radioactivity of the samples is measured on Scintillation counter (Topcount, HP). The IC50 values of different peptides were calculated using GraFit software.

5 Alternatively, CDK2/cyclin A kinase assays may be performed in 96-well plates using recombinant CDK2/cyclin A. Assay buffer consisted of 25 mM β -glycerophosphate, 20 mM MOPS, 5 mM EGTA, 1 mM DTT, 1mM NaVO₃, pH 7.4, into which is added 2 – 4 μ g of CDK2/cyclin A with substrate pRb(773-928). The reaction is initiated by addition of Mg/ATP mix (15mM MgCl₂, 100 μ M ATP with 30-50 kBq per well of 10 $[\gamma$ -³²P]-ATP) and mixtures incubated for 10 – 30 min, as required, at 30 °C. Reactions were stopped on ice, followed by filtration through p81 filterplates (Whatman Polyfiltrronics, Kent, UK). After washing 3 times with 75 mM orthophosphoric acid, plates were dried, scintillant added and incorporated radioactivity measured in a scintillation counter (TopCount , Packard Instruments, Pangbourne, Berks, UK).

15 PKC α kinase activity may be measured by the incorporation of radio-labeled phosphate in Histone 3, as described. The reaction mixture (total volume 65 μ l) consist of 50 mM Tris-HCl, 1 mM Calcium acetate, 3 mM DTT, 0.03 mg/ml Phosphatidylserine, 2.4 μ g/ml PMA, 0.04% NP40, 12 mM Mg/Cl, purified PKC α - 20 100 ng, Histone 3, 0.2mg/ml, 100 μ M ATP, 0.2 μ Ci $[\gamma$ -³²P]-ATP. The reaction is carried over 15 min at 37°C in microplate shaker and is stopped by adding 10 μ l 75 mM orthophosphoric acid and placing the plate on ice. 50 μ l of the reaction mixture is transferred onto P81 filterplate and after washing off the free radioactive phosphate (3 times with 200 μ l 75 mM orthophosphoric acid per well) 50 μ l of scintillation liquid (Microscint 40) were added to each well and the radioactivity is measured on 25 Scintillation counter (Topcount, HP).

For use in said assays CDK2 and/or PKC may be obtained from available sources or produced by recombinant methods as described. His-tagged CDK2/Cyclin E and

CDK1/Cyclin B may be co-expressed and PKC α singularly expressed in Sf 9 insect cells infected with the appropriate baculovirus constructs. The cells are harvested two days after infection by low speed centrifugation and the proteins purified from the insect cell pellets by Metal-chelate chromatography. Briefly, the insect cell pellet is lysed in Buffer A (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.02% NP40 and 5 mM β -mercaptoethanol, 1 mM NaF, 1 mM Na₃VO₄ and Protease Inhibitors Coctail (Sigma) containing AEBSF, pepstatin A, E 64, bestatin, leupeptin) by sonication. The soluble fraction is cleared by centrifugation and loaded onto Ni-NTA-Agarose (Quiagen). Non bound proteins were washed off with 300 mM NaCl, 5-15 mM Imidazole in Buffer A and the bound proteins eluted with 250 mM Imidazole in Buffer A. The purified proteins are extensively dialyzed against Storage buffer (20 mM HEPES pH 7.4, 50 mM NaCl, 2 mM DTT, 1 mM EDTA, 1 mM EGTA, 0.02% NP40, 10% v/v Glycerol) aliquoted and stored at -70°C. PKC- α - 6 x His may be purified the same way but using different buffers- 50 mM NaH₂PO₄, pH 8.0 and 0.05% Triton X-100 instead of Tris and NP40 respectively.

The results in the Table 1 below show that the compounds in question exhibit a high degree of selectivity for inhibition of CDKs.

20 **Table 1**

Compound	CDK2/cyclin E	Compound	CDK2/cyclin E
1	1.0 \pm 0.7	9	1.4
2	0.04	10	3.5
3	0.5	11	2.9
4	0.7	12	6.5
6	1.3 \pm 0.4	15	0.9
7	1.7	16	5.4
8	1.3		

Example 4Anti-proliferative effect of selected compounds

Selected compounds from the above examples were subjected to a standard cellular proliferation assay using a range of different human tumour cell lines. Standard 72-h

5 MTT (thiazolyl blue; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assays were performed (Haselsberger, K.; Peterson, D. C.; Thomas, D. G.; Darling, J. L. *Anti Cancer Drugs* 1996, 7, 331-8; Loveland, B. E.; Johns, T. G.; Mackay, I. R.; Vaillant, F.; Wang, Z. X.; Hertzog, P. J. *Biochemistry International* 1992, 27, 501-10). Human tumour cell lines were obtained from the ATCC (American Type Culture

10 Collection, 10801 University Boulevard, Manessas, VA 20110-2209, USA).

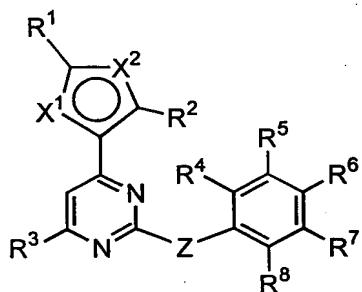
The results in Table 2 below illustrate the anti-proliferative effect of compounds described in this application.

15 **Table 2**

Compound	A549	Compound	A549
1	22 ± 13	11	60 ± 0.8
2	3.3 ± 2.7	12	67 ± 12
6	20.4 ± 0.2	13	37 ± 21
8	21 ± 13	14	18 ± 4
9	18 ± 4	15	15 ± 4
10	39 ± 18	16	17 ± 5

CLAIMS

1. A compound of general formula I:



wherein:

one of X¹ and X² is NH and the other of X¹ and X² is CR⁹;

10

Z is NH, NHCO, NHSO₂, NHCH₂, CH₂, CH₂CH₂, or CH=CH;

15

R¹, R², R³ and R⁹ are independently H, alkyl, aryl, aralkyl, heterocycle, halogeno, NO₂, CN, OH, alkoxy, aryloxy, NH₂, NH-R', N-(R')(R''), NH-aryl, N-(aryl)₂, COOH, COO-R', COO-aryl, CONH₂, CONH-R', CON-(R')(R''), CONH-aryl, CON-(aryl)₂, SO₃H, SO₂NH₂, CF₃, CO-R', or CO-aryl, wherein alkyl, aryl, aralkyl and heterocycle groups may be further substituted with one or more groups selected from halogeno, NO₂, CN, OH, O-methyl, NH₂, COOH, CONH₂ and CF₃;

20

R⁴, R⁵, R⁶, R⁷, and R⁸ are independently from each other H, substituted or unsubstituted lower alkyl, halogeno, NO₂, CN, OH, substituted or unsubstituted alkoxy, NH₂, NH-R', N-(R')(R''), COOH, COO-R', CONH₂, CONH-R', CON-(R')(R''), SO₃H, SO₂NH₂, or CF₃;

wherein R' and R'' are each independently alkyl groups that may be the same or different;

with the proviso that when R¹ and R² are H, X¹ is NH, X² is CH, and R³ is H, the phenyl group is not

5 unsubstituted,

4-ethyl,

3-methyl,

3-(1,1,2,2-tetrafluoroethoxy),

3,4,5-trimethoxy,

10 when the other groups R⁴-R⁸ are H;

and pharmaceutically acceptable salts thereof.

2. A compound according to claim 1, wherein;

15 - X¹ and X² are CR⁹ and NH respectively;

- R¹, R², R³ and R⁹ are each independently selected from H, alkyl, aryl, aralkyl, halogeno, NO₂, CN, OH, alkoxy, aryloxy, NH₂, NH-R', N-(R')(R''), COOH, COO-R', CONH₂, CONH-R', CON-(R')(R''), SO₃H, SO₂NH₂, CF₃, and CO-R' wherein 20 alkyl, aryl and aralkyl groups may be further substituted with one or more groups selected from halogeno, NO₂, CN, OH, O-methyl, NH₂, COOH, CONH₂ and CF₃;

- Z is selected from N, NHSO₂ and NHCH₂;

25 - R⁴-R⁸ are each independently selected from H, halogeno, nitro, amino, alkoxy, carbamoyl, sulfamyl, C₁₋₄ alkyl and substituted C₁₋₄ alkyl.

3. A compound according to any preceding claim, wherein Z is NH and R³ is H.
4. A compound according to any preceding claim wherein R¹ is H.
5. A compound according to any preceding claim wherein R¹, R² and R⁹ are each independently H, halogeno or C₁₋₄ alkyl groups.
6. A compound according to claim 5 wherein R¹ is H, and R² and R⁹ are both chloro or methyl.

10

7. A compound according to any preceding claim wherein R⁴ to R⁸ are selected independently from H, F, NH₂, NO₂, OH, Cl, Br, I, CN, CH₂OH, CF₃ and OMe.
8. A compound according to any preceding claim selected from 2-[N-(phenyl)]-4-(2,4-dimethylpyrrol-3-yl)pyrimidineamines in which the phenyl group is 2-, 3- or 4-substituted by at least one of H, F, NH₂, NO₂, OH, Cl, Br, I, CN, CH₂OH, CF₃ or OMe.
9. A compound according to claim 8, wherein the phenyl group is mono-substituted by F, NH₂, NO₂, OH, Cl, Br, I, CH₂OH, CN, CF₃ or OMe at any of the 2,3 or 4-positions, or di-substituted by 2,4-difluoro, 3,5-difluoro, 3,4-difluoro, 2,4-dichloro, 3,5-dichloro, 3,4-dichloro or 4-chloro-3-trifluoromethyl.

10. A compound according to claim 1 selected from;
25 {3-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-phenyl}-methanol,
3-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-benzonitrile,
4-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-benzonitrile,
[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(4-fluoro-phenyl)-amine,
[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(3-nitro-phenyl)-amine,

[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(4-iodo-phenyl)-amine,
(3,4-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
(4-Chloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
(3,5-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
5 4-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-phenol,
3-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-phenol,
(2,4-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
(2,4-Dichloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
(4-Chloro-3-trifluoromethyl-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-
10 yl]-amine,
[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(4-trifluoromethyl-phenyl)-amine,
[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(3-trifluoromethyl-phenyl)-amine,
(3-Chloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine.
15 11. A compound according to any preceding claim selected from the following:
[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(4-fluoro-phenyl)-amine,
[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(3-nitro-phenyl)-amine,
[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(4-iodo-phenyl)-amine,
4-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-benzonitrile,
20 (3,4-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
(3-Chloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
(3,5-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
3-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-phenol,
(4-Chloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
25 4-[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-ylamino]-phenol,
(2,4-Difluoro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine,
[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(4-trifluoromethyl-phenyl)-amine,
and

[4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(3-trifluoromethyl-phenyl)-amine.12. A compound according to claim 10 selected from the following: (2,4-Dichloro-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine, and (4-Chloro-3-trifluoromethyl-phenyl)-[4-(2,4-dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-amine.

13. The compound of claim 10 that is [4-(2,4-Dimethyl-1H-pyrrol-3-yl)-pyrimidin-2-yl]-(3-nitro-phenyl)-amine.

10 14. Pharmaceutical compositions comprising a compound as defined in any of claims 1 to 13 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable excipient.

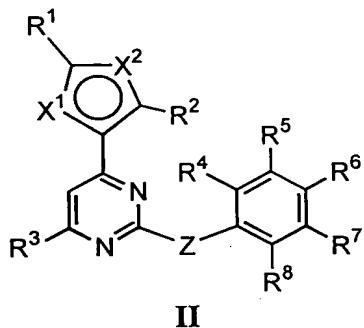
15 15. Use of a compound as defined in any of claims 1 to 13 or a pharmaceutically acceptable salt thereof in the treatment of a proliferative disorder.

16. Use according to claim 15, wherein the proliferative disorder is cancer or leukaemia.

20 17. Use according to claim 15 or 16, wherein said compound is administered in an amount sufficient to inhibit at least one CDK enzyme.

18. Use according to claim 17, wherein the CDK enzyme is CDK2 and/or CDK4.

19. Use of a compound of formula



5

wherein:

one of X¹ and X² is NH and the other of X¹ and X² is CR⁹;10 Z is NH, NHCO, NHSO₂, NHCH₂, CH₂, CH₂CH₂, or CH=CH;

R¹, R², R³ and R⁹ are independently H, alkyl, aryl, aralkyl, heterocycle, halogeno, NO₂, CN, OH, alkoxy, aryloxy, NH₂, NH-R', N-(R')(R''), NH-aryl, N-(aryl)₂, COOH, COO-R', COO-aryl, CONH₂, CONH-R', CON-(R')(R''), CONH-aryl, CON-(aryl)₂,
 15 SO₃H, SO₂NH₂, CF₃, CO-R', or CO-aryl, wherein alkyl, aryl, aralkyl and heterocycle groups may be further substituted with one or more groups selected from halogeno, NO₂, CN, OH, O-methyl, NH₂, COOH, CONH₂ and CF₃;

20 R⁴, R⁵, R⁶, R⁷, and R⁸ are independently from each other H, substituted or unsubstituted lower alkyl, halogeno, NO₂, CN, OH, substituted or unsubstituted alkoxy, NH₂, NH-R', N-(R')(R''), COOH, COO-R', CONH₂, CONH-R', CON-(R')(R''), SO₃H, SO₂NH₂, or CF₃;

25 wherein R' and R'' are each independently alkyl groups that may be the same or different;

with the proviso that when R^1 and R^2 are H, X^1 is NH, X^2 is CH, and R^3 is H, the phenyl group is not

3-(1,1,2,2-tetrafluoroethoxy), or

3,4,5-trimethoxy,

5 when the other groups R^4 - R^8 are H;

and pharmaceutically acceptable salts thereof;

in the manufacture of a medicament for use in the treatment of a proliferative disease.

20. Use according to claim 19, wherein the compound is as defined in any of
10 claims 2 to 13.

21. Use according to any one of claims 15 to 20 wherein said compound is
administered in combination with one or more other anticancer compounds.

ABSTRACT

The present invention relates to 2-substituted 4-heteroaryl-pyrimidines, their preparation, pharmaceutical compositions containing them and their use as inhibitors of cyclin-dependent kinases (CDKs) and hence their use in the treatment of proliferative disorders such as cancer, leukaemia, psoriasis and the like.

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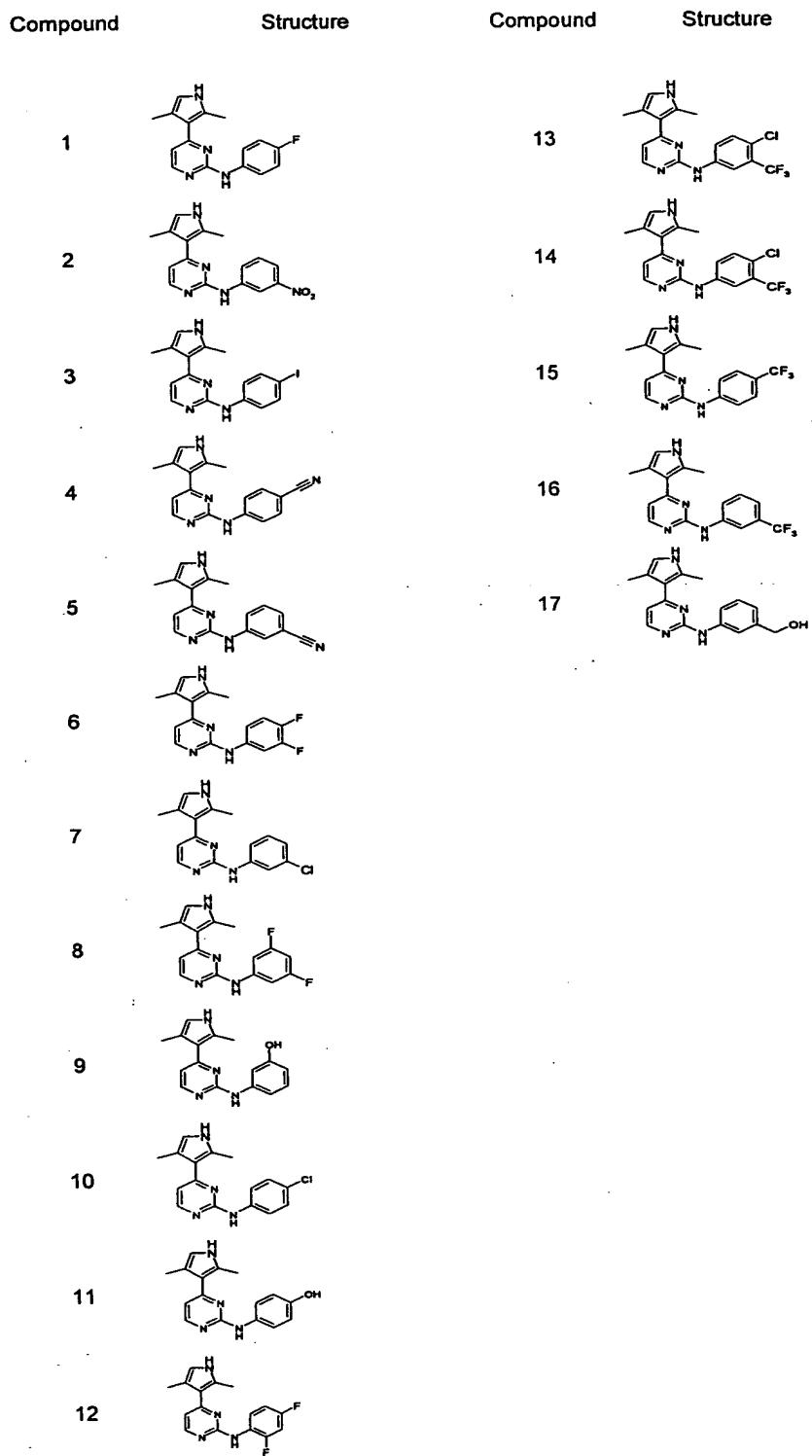


Figure 1

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